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In vitro Fermentation Characteristics and Rumen Microbial Population of Diet Supplemented with *Saccharomyces cerevisiae* and Rumen Microbe Probiotics

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ABSTRACT

The objective of this study was to select three strains of probiotic *Saccharomyces cerevisiae* and to evaluate the effect of *S. cerevisiae* and rumen bacteria isolate (MR4) supplementation and their combination on rumen fermentability and rumen microbial population. Experiment 1 was designed in a 4 x 5 factorial randomized block design with 3 replications. The first factor was *S. cerevisiae* strain consisted of control treatment (without *S. cerevisiae* supplementation), NBRC 10217, NRRL Y 567 and NRRL 12618, and the second factor was incubation time consisted of 0, 1, 2, 3, and 4 h. Ration was basal ration for feedlot with forage to concentrate ratio (F:C)= 60:40. Dosage of each treatment with *S. cerevisiae* was 5 x 10^{10} cfu/kg ration. Experiment 2 was designed in randomized block design with 4 treatments: P0= basal ration of feedlot; P1= P0 + *S. cerevisiae*; P2= P0 + MR4 isolate (5 x 10^{7} cfu/kg ration); P3= P0 + *S. cerevisiae* and MR4 isolate. The result of experiment 1 showed that supplementation of *S. cerevisiae* NRRL 12618 had the highest *S. cerevisiae* population and increased rumen bacterial population. This strain was selected as probiotic in experiment 2. The result from experiment 2 showed that probiotic supplementation stabilized rumen pH and produced the highest NH_3 concentration (P<0.05) and bacterial population (P<0.05). As compared with control, all treatments reduced protozoa population (P<0.05). Combination of *S. cerevisiae* and MR4 probiotics produced the highest total volatile fatty acids (VFA) and isovalerate (P<0.05). It was concluded that strain *S. cerevisiae* NRRL 12618 had potential as probiotic yeast. Supplementation with this strain increased fermentability, rumen isoacid and decreased A:P ratio. Those abilities could be improved with MR4 rumen isolate probiotic.

Key words: probiotic, fermentation characteristics, rumen microbe, Saccharomyces cerevisiae

ABSTRAK

Penelitian bertujuan untuk menyeleksi tiga strain probiotik *Saccharomyces cerevisiae,* serta mengevaluasi pengaruh suplementasi probiotik *S. cerevisiae* dan isolat mikroba rumen (MR4) dan kombinasinya pada fermentabilitas dan populasi mikroba rumen secara *in vitro*. Percobaan dilakukan dalam dua tahap, Percobaan 1 dirancang dengan rancangan acak kelompok faktorial 4 x 5 dengan 3 ulangan. Faktor pertama adalah strain *S. cerevisiae* yang terdiri atas kontrol (tidak disuplementasi dengan *S. cerevisiae*), NBRC 10217, NRRL Y 567 and NRRL 12618, dan faktor kedua adalah waktu inkubasi, yaitu 0, 1, 2, 3, dan 4 jam. Ransum yang digunakan adalah ransum basal sapi pedaging dengan rasio H:K= 60:40. Dosis *S. cerevisiae* yang diberikan adalah 5 x 10^{10} cfu/kg ransum. Percobaan 2 dirancang dengan rancangan acak kelompok dengan 4 perlakuan, yaitu: P0=ransum basal untuk sapi feedlot; P1= P0 + *S. cerevisiae*; P2= P0 + isolat MR4 (dosis 5 x 10^{7} cfu/kg ransum); dan P3= P0 + *S. cerevisiae* dan isolat MR4. Hasil percobaan 1 menunjukkan bahwa suplementasi *S. cerevisiae* NRRL 12618 memiliki laju pertumbuhan *S. cerevisiae* 3,3%/jam. Strain ini terpilih sebagai *probiotic yeast* pada percobaan 2. Hasil percobaan 2 menunjukkan bahwa suplementasi probiotik masih dapat mempertahankan pH rumen normal, sehingga pH rumen lebih stabil dan berdampak pada peningkatan konsentrasi NH_3 (P<0,05), populasi bakteri rumen total (P<0,05) dan penurunan populasi protozoa (P<0,05). Kombinasi probiotik *S. cerevisiae* dan MR4 mampu meningkatkan konsentrasi *volatile fatty acid* (VFA) total dan isovalerat (P<0,05). Strain *S. cerevisiae* NRRL 12618 memiliki potensi untuk digunakan sebagai *probiotic yeast*. Suplementasi dengan strain ini mampu meningkatkan fermentabilitas dan kandungan *isoacid* dalam rumen serta menurunkan rasio A:P. Kemampuan ini dapat ditingkatkan jika probiotik *S. cerevisiae* dikombinasikan probiotik MR4.

Kata kunci: karakteristik fermentasi, probiotik, mikroba rumen, Saccharomyces cerevisiae

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INTRODUCTION

The efforts to improve productivity and quality of beef cattle are still constrained by nutrition problem. The cattle kept in feedlot consume large amounts of concentrate compared to the amount of forage. High concentrate consumption can improve the accumulation of lactic acid by rumen bacteria and causing acidosis. Nutritionist has focused on the modification of rumen fermentation to improve productivity. Consequently, research has involved of diet such as supplementation with feed additive that have potential to modify the rumen environment (Calsamiglia *et al.,* 2006). One of feed additive is probiotic. Probiotics are defined as live microbial feed supplements that have beneficial effect on the host animal by improving microbial balance in digestive tract. Supplementation with probiotic was aimed to increase the capacity of animal digestion. Probiotic in ruminant is used to modify the microorganism ecosystem and rumen fermentation. This method can be accomplished by manipulating microbial population to stimulate fiber and starch digestibility, improving volatile fatty acid (VFA) production and reduce lactate accumulation that can reduce rumen pH.

One of microorganism that potential to serve as probiotic is *Saccharomyces cerevisiae*. Supplementation with live *S. cerevisiae* has been associated with stabilizing rumen pH through promotion of the use of lactic acid by lactate-utilizing bacteria (Seo *et al.,* 2010), and competition with rumen bacteria for rapidly fermentable carbohydrate (Bach *et al.,* 2007). *S. cerevisiae* cells are believed to eliminate trace of oxygen in the rumen thus helping oxygen-sensitive bacteria to grow (Marden *et al.,* 2008). The effect of *S. cerevisiae* on this rumen microbes activities are vary depending on the type of strain used (Sullivan & Bradford, 2011). Therefore, it is necessary to select some *S. cerevisiae* that can grow in the rumen fluid and stimulates the activity of rumen microbes. The ability of *S. cerevisiae* as probiotics can be improved by combining *S. cerevisiae* with rumen microbe isolates called MR4 isolate. MR4 isolate is anaerobic rumen microbe that has ability to increase population of rumen microbes. The objective of this study was to select three strains of probiotic *S. cerevisiae* and to evaluate the effect of *S. cerevisiae* and rumen bacteria isolate (MR4) supplementation and their combination on rumen fermentability and rumen microbial population.

MATERIALS AND METHODS

Preparation of *Saccharomyces cerevisiae* Probiotic

S. cerevisiae strain NBRC 10217 was obtained from Biotechnology Laboratory, Indonesian Institute of Science. Strain NRRL Y 567 and NRRL 12618 were obtained from IPB Culture Collection (IPBCC). The strain was grown on potato dextrose broth (PDB, Difco) and incubated for 48 h at 30°C. Pure culture of *S. cerevisiae* was added into rice flour with ratio 5 mL culture : 20 g. The mixture is stirred until homogenized and incubated for 24 h at 30°C. The population of *S. cerevisiae* was measured by bacteriological analytical manual (BAM).

Encapsulation of MR4 Isolate Probiotic

Encapsulation of MR4 isolate was modified based on Krasaekoopt *et al.* (2003). One hundred milliliter of Na-alginate 2% solution was added with 100 mL of 2% starch solution. The solution was mixed with MR4 culture that was grown in BHI medium while flowing in CO_2. Then 200 mL of canola oil containing 0.2 mL lecithin was added into solution and homogenized for 20 min. Two hundred milliliters of 0.1 M $CaCl_2$ solution was added slowly. Oil and water were separated, and the beads were harvested by filtration. The beads were washed with 0.9% saline solution containing 5% glycerol. The beads were mixed with skim milk powder.

Experiment 1: Selection of *Saccharomyces cerevisiae* as Probiotic *in Vitro*

The purpose of this study was to select *S. cerevisiae* strain that capable of living in rumen fluid and stimulating the growth of rumen bacteria. The experiment was designed in 4 x 5 factorial randomized block design with 3 replications. The first factor was *S. cerevisiae* strain consisted of 4 treatments (without SC supplementation as control treatment, NBRC 10217, NRRL Y 567 and NRRL 12618) and the second factor was time of incubation consisted of 5 levels (0, 1, 2, 3, and 4 h). *In vitro* fermentation was conducted according to the method of Tilley & Terry (1963). Into each fermentation tube, 500 mg substrate, 40 mL McDougall buffer, and 10 mL rumen fluid were added. Fermenter tubes were flowed by CO_2 for 30 s (pH 6.5-6.9) and incubated in shaker water bath at temperature of 39°C.

The ration contained 60% king grass forage and 40% concentrate mixture (cassava by product, rice bran, corn, groundnut meal, palm kernel meal, $CaCO_3$, and NaCl) (Table 1). Dosage of *S. cerevisiae* probiotic was 10^{10} cfu/kg ration. Rumen fluid was collected after 3 h feeding from the rumen of fistulated-Ongole crossbred cattle. After 0, 1, 2, 3, and 4 h, rumen fluid samples were collected for measurement of population of *S. cerevisiae* and rumen bacteria.

Experiment 2: Rumen Fermentability and Microbial Population

The best strain from Experiment 1 was used as probiotic *S. cerevisiae* in Experiment 2 and combined with MR4 isolate. The experiment was designed in randomized block design with 4 treatment consisted of: P0= basal feed for feedlot; P1= P0 + *S. cerevisiae* probiotic; P2= P0 + MR4 isolate (10^7 cfu/g ration); P3= P0 + probiotic *S. cerevisiae* + MR4 isolate. *In vitro* fermentation was conducted according to the method of Tilley & Terry (1963). After 4 h of incubation, rumen fluid sample was collected for NH_3, VFA, protozoa and rumen bacterial population.

Sampling and Measurement

Population of *S. cerevisiae* was measured by bacteriological analytical manual (BAM). Population of ru-

Table 1. Ingredient and nutrient composition of basal ration

Item	Amount
Forage : Concentrate	60:40
Concentrate	
Cassava by product (%)	30.00
Corn (%)	17.00
Rice bran (%)	28.00
Palm meal (%)	12.00
Nut meal (%)	10.00
NaCl (%)	1.00
$CaCO_3$ (%)	1.50
Buffer mineral (%)[a]	0.50
Nutrient composition[b]	
Dry matter (%)	91.48
Ash (% DM)	9.84
Crude protein (% DM)	10.00
Ether extract (% DM)	3.00
Crude fiber (% DM)	31.54
Non-structural carbohydrate (NSC)[c] (% DM)	45.62

Note:
The requirement of beef cattle was based on Lalman (2001) with BW= 346.5 kg and daily gain 1 kg/head/d.
[a]Buffer mineral content were Ca 15%, P 11%, Cl 12%, Na 8%, Mg 1.7%, Fe 250 ppm, Mn 138 ppm, Cu 100 ppm, Zn 65 ppm, I 15 ppm, Co 2 ppm, vitamin A 80000 IU/kg, vitamin D 25000 IU/kg, and vitamin E 1000 IU/kg.
[b]Chemical composition was analyzed in Research Center for Biological Resources and Biotechnology, Bogor Agricultural University (2015).
[c]NSC= 100 – (ash + crude protein + ether extract + crude fiber)

men bacteria were quantified by Ogimoto & Imai (1981) method that used BHI medium. Growth rate of *S. cerevisiae* was measured by equation $Pt = Po.e^{kt}$ (Pt= population in t hours, Po= population in 0 hours, k= constant of growth rate, and t= incubation time). The rumen pH was measured with pH meter. Concentration of NH_3 was measured by Micro-diffusion Conway method (General Laboratory Procedures 1966). Total VFA concentration and molar proportions of VFA were analyzed by using gas chromatography (GC 8A, Shimadzu Crop, Kyoto, Japan, capillary column type containing 10% SP-1200, 1% H_3PO4 on 80/100 Cromosorb WAW and nitrogen as gas carrier) (General Laboratory Procedures 1966). Protozoa populations were counted with *Fuch Rosenthal Counting Chamber* (4 x 4 x 0.2 mm) under a microscope (10 × 10) (Ogimoto & Imai, 1981).

Statistical Analysis

Data were tested by using Analysis of Variance (ANOVA) and the differences among treatments means were examined in Experiment 1 by Duncan's multiple range and in Experiment 2 by contrast orthogonal (Mattjik & Sumertajaya, 2006).

RESULTS AND DISCUSSION

Experiment 1: Selection of *Saccharomyces cerevisiae* as Yeast Probiotic *in Vitro*

The result of Experiment 1 showed that there was interaction between *S. cerevisiae* strain and incubation time on *S. cerevisiae* population in rumen fluid (P<0.05). The treatment was NRRL 12618 in 4 hours incubation time. All of *S. cerevisiae* strain could grow in rumen fluid with different growth rates (Figure 1). NRRL 12618 strain showed the highest growth rate in rumen fluid with growth rate was 3.3%/h. The growth rate of NBRC 10217 and NRRL Y 567 strains in rumen fluid were 2.2%/h and 2.3%/h, respectively. There was no *S. cerevisiae* growth on control treatment. It showed that *S. cerevisiae* counted in the rumen fluid originated from *S. cerevisiae* added in the beginning of incubation.

All of *S. cerevisiae* strains had the increased growth rate with the increased duration of incubation time. However, an increase in the growth rate of *S. cerevisiae* after 4 h was not yet known. The increasing population of *S. cerevisiae* in rumen fluid was caused by higher oxygen concentration in media, since one of the factors that affect the *S. cerevisiae* growth in rumen fluid was availability of oxygen in the rumen. Some of *S. cerevisiae* strain was adapted to anaerobic condition. This suggests that all strains are facultative anaerobe. Kawas *et al.* (2007) suggested that varying responses of yeast supplementation was attributed to the strain of the yeast and diet. Jurkovich *et al.* (2014) reported that the different yeast strains seemed to have different metabolic activities that were indicated by the different abilities to consume oxygen.

The effect of *S. cerevisiae* supplementation on the growth of rumen bacteria was presented in Figure 2. There was no interaction between *S. cerevisiae* strain and incubation time on the growth of rumen bacteria (P>0.05). Based on regression analysis, all treatment did not show the pattern of linear, quadratic and cubic growth (P>0.05). Based on mean growth, there were tendency that *S. cerevisiae* supplementation increased rumen bacteria, while control treatment decreased rumen bacteria. This result was caused by the fact that there was no *S. cerevisiae* supplementation that could reduce CO_2 concentration during incubation *in vitro* that eventually decreased the activities of rumen bacteria. Treatment with *S. cerevisiae* strain NRRL 12618 had highest rumen bacteria population, as compared to *S. cerevisiae* strain NBRC 10217 and *S. cerevisiae* strains NRRL Y-567. Based on the highest growth rate in rumen fluid and the ability to stimulate rumen bacteria then *S.*

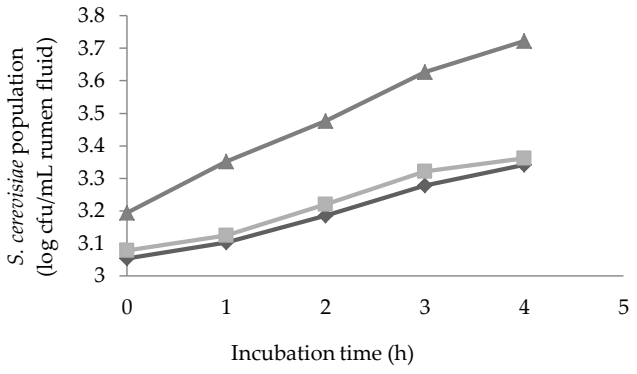

Figure 1. The growth of three *S. cerevisiae* strains (NBRC 10217, -♦-; NRRL Y 567, -■-; NRRL 12618, -▲-) in rumen fluid

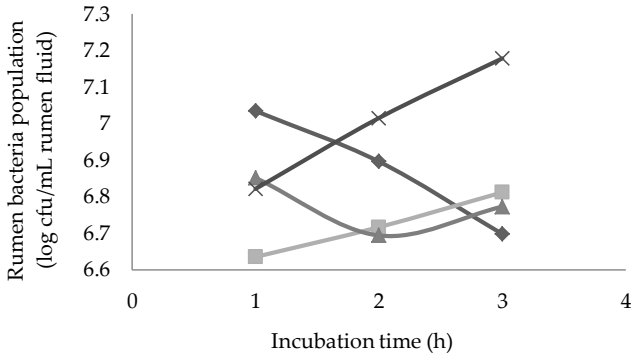

Figure 2. The growth of rumen bacteria supplemented with three *S. cerevisiae* strains (Control, -♦-; NBRC 10217, -■-; NRRL Y 567, -▲-; NRRL 12618, -×-)

cerevisiae strain NRRL 12618 was selected as probiotic yeast that would be used in Experiment 2.

The increase in population of rumen bacteria in the treatment of *S. cerevisiae* supplementation was caused by the ability of the *S. cerevisiae* to eliminate oxygen in the rumen to maintain its activity (Seo *et al.*, 2010). Chaucheryas-Durand *et al.* (2008) reported that *S. cerevisiae* supplementation in ration could reduce redox potential of the rumen that eventually provided a better ecological condition for the growth and activity of anaerobic microorganisms in the rumen. Low redox potential stimulated the attachment of fibrolytic bacteria to cellulose particles and increased the initial rate of cellulolysis (Pinloche *et al*., 2013). Other factors that lead to high populations of rumen bacteria in *S. cerevisiae* supplementation treatment were the abilities of *S. cerevisiae* to produce and provide the growing factors such as organic acids and vitamins that eventually stimulate the growth of rumen bacteria population. Yeast supplementation significantly increased the relative occurrence of some rumen bacteria such as *Megasphaera, Ruminococcus, Eubacterium, Selenomonas* and *Bifidobacterium* and there was a tendency for Fibrobacter (Pinloche *et al.*, 2013). It showed that live yeast supplementation increased the number and activity of bacterial population in the rumen.

Experiment 2: Rumen Fermentability and Microbial Population

Characteristics of rumen fermentation and microbial population were shown in Table 2. There was no different in rumen pH during 4 h incubation between control and probiotic supplementation. There was a tendency that probiotic *S. cerevisiae* had ability to maintain rumen pH. It showed that probiotic supplementation could maintain normal pH with averages of 6.8-7. Rumen pH was in normal range and could stimulate rumen bacteria population. Yeast is able to compete against amylolytic bacteria that could prevent lactic acid accumulation in the rumen (Erasmus *et al*., 2005). Thrune *et al.* (2009) reported that dairy cow supplemented with yeast spent less in low pH (pH<5.4) as compared to control.

Probiotic supplementation increased the concentration of ammonia (NH_3) in the rumen (P<0.05). The ability of single probiotic in producing NH_3 in the rumen was similar to combination probiotics (P>0.05), while mean of NH_3 concentration on P2 treatment was higher than P1 treatment i.e., 6.49 mM and 5.95 mM, respectively. The lower concentration may be due to competition between yeast and the bacteria cells for energy supply and by direct inhibitory effect of yeast on small peptides and bacterial peptidases (Chaucheyras-Durand-Durand *et al.,* 2008). Rohilla *et al.* (2009) reported that NH_3-N was efficiently utilized by the addition of yeast in the ration. The low value of NH_3 in the rumen is influenced by the low level of protein in the diet. NH_3 is used as a source of N for microbial protein synthesis (Bach *et al.,* 2005) so that the bacteria can grow in the rumen. The result showed that supplementation of single or combined probiotic could increase NH_3 concentration in the rumen. NH_3 is used for microbial protein synthesis to increase supply of post-rumen amino acids that are beneficial for the host.

The increase in NH_3 concentration in P2 was assumed to be caused by the higher bacteria population. The NH_3 concentration in this study was in line with the rumen bacteria population. Probiotic supplementation increased the populations of total rumen bacteria (P<0.05). The ability of single (*S. cerevisiae* or MR4) culture of probiotic was equal to that of co-culture probiotic (combination *S. cerevisiae* and MR4) in stimulating the growth of bacteria. The population of bacteria in P2 was higher than that of in P1 treatment i.e., 7.97 log cfu/mL and 7.29 log cfu/mL, respectively. The highest population of rumen bacteria on P2 was caused by the fact that MR4 isolate itself is rumen bacteria. Malik & Singh (2009) reported that yeast supplementation increased population of cellulose-degrading bacteria in the rumen. These results indicated that when conditions in the rumen were stabilized, there was an increase in the rate of fiber degradation through the increased activities of cellulolytic bacteria.

Probiotic supplementation decreased protozoa population in rumen during 4 h incubation time (P<0.05). The decreased population of protozoa in the group supplemented with probiotic was caused by the

Table 2. Fermentation characteristic and rumen microbe population *in vitro* from beef cattle supplemented with *S. cerevisiae* and rumen bacteria isolate (MR4) probiotics

Variable	Treatments			
	P0	P1	P2	P3
pH	6.88±0.04	6.96±0.15	6.88±0.04	6.92±0.11
NH_3 (mM)	5.73±0.30^{c}	5.95±0.77^{b}	6.49±0.23^{a}	6.26±0.18^{b}
Rumen bacteria (log cfu/mL)	7.04±0.76^{c}	7.29±0.81^{b}	7.97±0.47^{a}	7.67±0.66^{b}
Protozoa (log sel/mL)	3.98±0.11^{a}	3.79±0.13^{b}	3.80±0.14^{b}	3.78±0.23^{b}

Note: P0 (basal ration); P1 (P0 + *S. cerevisiae* probiotic), P2 (P0 + MR4 probiotic); P3 (P0 + *S. cerevisiae* probiotic + MR4 probiotic). Means in the same row with different superscripts differ significantly (P<0.05).

Tabel 3. Fermentation characteristic and rumen microbe population *in vitro* from beef cattle supplemented with *S. cerevisiae* and rumen bacteria isolate (MR4) probiotics

Variable	Treatments			
	P0	P1	P2	P3
Total VFA (mM)	101.05±5.41[b]	115.53±17.88[a]	110.36±0.88[a]	119.89±5.41[a]
Acetate (mM)	50.46±3.39	58.15±7.12	52.80±0.85	55.82±2.49
Propionate (mM)	34.71±3.54	43.31±7.42	36.17±1.75	38.93±2.39
Butyrate (mM)	9.60±0.43	12.51±2.01	10.77±0.42	12.16±1.06
Isobutyrate (mM)	6.13±1.14	6.50±1.04	6.66±0.45	7.26±0.74
Valerate (mM)	0.66±0.21	1.04±0.63	0.71±0.25	1.03±0.05
Isovalerate (mM)	1.98±0.87[c]	1.89±0.52[d]	2.79±0.96[b]	3.93±0.65[a]
Acetate : Propionate	1.46±0.05	1.35±0.10	1.46±0.08	1.43±0.05

Note: P0 (basal ration); P1 (P0 + *S. cerevisiae* probiotic), P2 (P0 + MR4 probiotic); P3 (P0 + *S. cerevisiae* probiotic + MR4 probiotic). Means in the same row with different superscripts differ significantly (P<0.05).

increased population of bacteria in the rumen fluid. The result of this observation showed that probiotic could be used as a defaunation agent. Kowalik *et al.* (2012) reported that supplementation of live *S. cerevisiae* decreased rumen protozoa, while supplementation of *S. cerevisiae* metabolite increased rumen protozoa. These results could be caused the availability of nutrients and metabolites released by the dead *S. cerevisiae* that could serves as prebiotic for the growth of rumen protozoa. *S. cerevisiae* contains soluble factors (vitamin B, amino acid, organic acids such as malic, fumaric and aspartate) and cell membrane of *S. cerevisiae* contains mannan and β-glucan that can stimulate the growth of *Entodinium*. Dobicki *et al.* (2006) reported that *S. cerevisiae* metabolite was able to increase population of *Diplodinium spp*. as compared with control treatment.

The effect of probiotic supplementation on VFA concentration was presented in Table 3. Probiotic supplementation increased total VFA concentration (P<0.05) but probiotic supplementation did not change molar proportion of acetate, propionate, and butyrate concentrations (P>0.05). Combination of *S. cerevisiae* and MR4 probiotic had the same effect as single probiotic on VFA total concentration. The increase in total VFA concentration in the rumen was caused by the stability of rumen pH that eventually stimulated the growth of bacterial population. Desnoyers *et al.* (2009) concluded that yeast supplementation increased rumen pH and volatile fatty acid concentration. Isobutyric acid concentration was not affected by probiotic supplementation (P>0.05). The growths of specific strains of fiber-digesting bacteria, which have major roles in the digestion of fiber to produced higher short chain fatty acid, were stimulated by yeast supplementation (Harikrishna *et al.* 2013). Probiotic supplementation increased isovalerate concentration (P<0.05). Combination of *S. cerevisiae* and MR4 probiotic increased isovalerate concentration (P<0.05). The high concentrations of isobutyrate and isovalerate showed the increased proteolytic activities in the group supplemented with *S. cerevisiae* so that proteolytic bacteria could utilize brach-chain amino acid as energy source to produce brach-chain fatty acid as the end product (Vyas *et al.,* 2014). Isoacid (isobutyrate and isovalerate) concentrations increased with the addition of yeast culture (Lascano & Heinrichs, 2009). Cellulolytic bacteria require isoacid from protein deamination to stimulate their growth and the capacity to degrade fiber. Probiotic supplementation had no effect on acetate to propionate ratio (A:P) (P>0.05). All treatment had low A:P ratios indicating that propiogenic activity was more dominant in feed degradation. Besides the effect of stimulating *S. cerevisiae* on VFA concentrations, *S. cerevisiae* supplementation changed the molar proportion of VFA in the rumen to increase the glucogenic potential of the diet (lower A:P ratio). Probiotic supplementation is more effective, resulting in a higher A:P ratio (Guedes *et al.,* 2008). The high concentration of propionate was caused by the high *Propionibacteria,* such as *Meganosphera elsdenii* and *Selenomonas ruminantium,* converting lactic acid into propionate (Silberberg *et al.,* 2013). Propionate is a precursor for gluconeogenesis process, so the increase in propionate in the rumen could improve glucose synthesis from propionate in the liver (Stein *et al.,* 2006).

CONCLUSION

Saccharomyces cerevisiae could grow in rumen fluid and increased rumen bacterial population. One of potential probiotic yeast was *S. cerevisiae* strain NRRL 12618. Combination of *S. cerevisiae* and rumen microbe probiotics increased rumen fermentability, isoacid concentration and decreased acetate : propionate ratio.

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